IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants:	Eijiro WATANABE et al.		
Serial No.:	08/992,914	Group:	1638
Filed:	December 18, 1997	Examiner:	D.H.Kruse
For:	RAFFINOSE SYNTHASE GENES AND THEIR USE		

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; http://www.sdc.co.jp/genetyx/) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:
MAPPSITKTATLQDVISTIDIGNGNSPLFSITLDQSRDFLANGHPFLTQV
PPNITTTTTTTASSFLNLKSNKDTIPNNNNTMLLQQGCFVGFNSTEPKSH
HVVPLGKLKGIKFMSIFRFKVWWTTHWVGTNGQELQHETQMLILDKNDSL
GRPYVLLLPILENTFRTSLQPGLNDHIGMSVESGSTHVTGSSFKACLYIH
LSNDPYSILKEAVKVIQTQLGTFKTLEEKTAPSIIDKFGWCTWDAFYLKV
HPKGVWEGVKSLTDGGCPPGFVIIDDGWQSICHDDDDEDDSGMNRTSAGE
QMPCRLVKYEENSKFREYENPENGGKKGLGGFVRDLKEEFGSVESVYVWH
ALCGYWGGVRPGVHGMPKARVVVPKVSQGLKMTMEDLAVDKIVENGVGLV
PPDFAHEMFDGLHSHLESAGIDGVKVDVIHLLELLSEEYGGRVELARAYY
KALTSSVKKHFKGNGVIASMEHCNDFFLLGTEAISLGRVGDDFWCSDPSG
DPNGTYWLQGCHMVHCAYNSLWMGNFIQPDWDMFQSTHPCAEFHAASRAI
SGGPIYVSDCVGNHNFKLLKSLVLPDGSILRCQHYALPTRDCLFEDPLHN
GKTMLKIWNLNKYTGVLGLFNCQGGGWCPEARRNKSVSEFSRAVTCYASP
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKLRLMKCSDRLKVSLEPFSFEL
MTVSPVKVFSKRFIQFAPIGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC
GEMSVFASEKPVCCKIDGVKVKFLYEDKMARVQILWPSSSTLSLVQFLF

Sc-03:
MAPSFSKENSKTCDEVANHDDCNTCPIISLEESNFMVNGHVILSQVPSNI
TAISKMGFDGLFVGFDAPEPKARHVVSVGQLKGIPFMSIFRFKVWWTTHW
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD
YVDICVESGSTKVVGDSFRAVLYIRAGPDPFKLIKDTMKEVQAHLGTFKL
LDDKTPPGIVDKFGWCTWDAFYLKVEXYGVWEGVKGLVENGVPPGLVLID
DGWQSICHDDDPITDQEGINRTSAGEQMPCRLIKYEENFKFRDYKSPNIM
GHEDHPNMGMRAFVRDLKEEFKTVEHVYVWHAFTGYWGGVRPNVPGLXEA
QVVTPKLSPGLEMTMEDLAVDKIVNNGIGLVQPDKAQELYEGLHSHLENC

GIDGVKVDVIHLLEMMAEDYGGRVELAKTYYKAITESVRKHFKGNGVIAS
MEQCNDFMLLGTETICLGRVGDDFWPTDPSGDINGTYWLQGCHMVHCAYN
SLWMGNFIIPDWDMFQSTHPCAEFHAASRAISGGPIYVSDVVGKHNIPLL
KRLVLADGSILRCEYHALPTKDCLFVDPLHDGKTMLKIWNLNKYNGVLGV
FNCQGGGWSRESRKNLCFSEYSKPISCKTSPKDVEWENGHKPFPIKGVEC
FAMYFTKEKKLILSQLSDTIEISLDPFDYELIVVSPMTILPWESIAFAPI
GLVNMLNAGGAVKSLDISEDNEDKMVQVGIKGAGEMMVYSSEKPKACRVN
GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYLF

Sc-04 (truncated):
MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS
LQPGLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM
HLGTFKLLEEKTAPVIIDKFGWCTWDAFYLKVHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAICHDEDPITDQEGMKRTSAGEQMPCRLVKLEENYKFRQ
YCSGKDSEKGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLYEGLHSRLES
AGIDGVKVDVIHLLEMLSEEYGGRVELAKAYYKALTASVKKHFKGNGVIA
SMEHCNDFFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY
NSLWMGNFIQPDWDMFQSTHPCAEFHAPLGPSLVDQFTLVIVLESTTSSC
SRASLCLMGRFCVVNTMHSPHETVCLKTPCMMGRQCSKFGISTNIQVFWV
YLIAKEVGGVP

Sc-05:
MAPPSVIKSDAAVNGIDLSGKPLFRLEGSDLLANGHVVLTDVPVNVTVTA
SPYLADKDGEPVDASAGSFIGFNLDGEPRSRHVASIGKLRDIRFMSIFRF
KVWWTTHWVGSKGSDIENETQIIILENSGSGRPYVLLLPLLEGSFRSSFQ
PGEDDDVAVCVESGSTQVTGSEFRQVVYVHAGDDPFKLVKDAMKVVRVHM
NTFKLLEEKXPPGIVDKFGWCTWDAFYLTVNPDGVHKGVKCLVDGGCPPG
LVLIDDGWQSIGHDSDGIDVEGMSCTVAGEQMPCRLLKFQENFKFRDYVS
PKDKNEVGMKAFVRDLKEEFSTVDYIYVWHALCGYWGGLRPGAPTLPPST
IVRPELSPGLKLTMQDLAVDKIVDTGIGFVSPDMANEFYEGLHSHLQNVG
IDGVKVDVIHILEMLCEKYGGRVDLAKAYFKALTSSVNKHFDGNGVIASM
EHCNDFMFLGTEAISLGRVGDDFWCTDPSGDINGTYWLQGCHMVHCAYNS
LWMGNFIQPDWDMFQSTHPCAEFHAASRAISGGPIYISDCVGQHDFDLLK
RLVLPDGSILRCEHYALPTRDRLFEDPLHDGKTMLKIWNLNKYTGIIGAF
NCQGGGWCRETRRNQCFSQCVNTLTATTNPKDVEWNSGNNPISVENVEEF
ALFLSQSKKLVLSGPNDDLEITLEPFKFELITVSPVVTIEGSSVQFAPIG
LVNMLNTSGAIRSLVYHEESVEIGVRGAGEFRVYASRKPASCKIDGEVVE
FGYEESMVMVQVPWSAPEGLSSIKYEF

PsRFS:

MAPPSITKTATQQDVISTVDIGNSPLLSISLDQSRNFLVNGHPFLTQVPP
NITTTTTSTPSPFLDFKSNKDTIANNNNTLQQQGCFVGFNTTEAKSHHVV
PLGKLKGIKFTSIFRFKVWWTTHWVGTNGHELQHETQILILDKNISLGRP
YVLLLPILENSFRTSLQPGLNDYVDMSVESGSTHVTGSTFKACLYLHLSN
DPYRLVKEAVKVIQTKLGTFKTLEEKTPPSIIEKFGWCTWDAFYLKVHPK
GVWEGVKALTDGGCPPGFVIIDDGWQSISHDDDDPVTERDGMNRTSAGEQ
MPCRLIKYEENYKFREYENGDNGGKKGLVGFVRDLKEEFRSVESVYVWHA
LCGYWGGVRPKVCGMPEAKVVVPKLSPGVKMTMEDLAVDKIVENGVGLVP
PNLAQEMFDGIHSHLESAGIDGVKVDVIHLLELLSEEYGGRVELAKAYYK
ALTSSVNKHFKGNGVIASMEHCNDFFLLGTEAISLGRVGDDFWCCDPSGD
PNGTYWLQGCHMVHCAYNSLWMGNFIHPDWDMFQSTHPCAEFHAASRAIS
GGPVYVSDCVGNHNFKLLKSFVLPDGSILRCQHYALPTRDCLFEDPLHNG
KTMLKIWNLNKYAGVLGLFNCQGGGWCPETRRNKSASEFSHAVTCYASPE
DIEWCNGKTPMDIKGVDVFAVYFFKEKKLSLMKCSDRLEVSLEPFSFELM
TVSPLKVFSKRLIQFAPIGLVNMLNSGGAVQSLEFDDSASLVKIGVRGCG
ELSVFASEKPVCCKIDGVSVEFDYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05:

MAPSFKNGGSNVVSFDGLNDMSSPFAIDGSDFTVNGHSFLSDVPENIVAS
PSPYTSIDKSPVSVGCFVGFDASEPDSRHVVSIGKLKDIRFMSIFRFKVW
WTTHWVGRNGGDLESETQIVILEKSDSGRPYVFLLPIVEGPFRTSIQPGD
DDFVDVCVESGSSKVVDASFRSMLYLHAGDDPFALVKEAMKIVRTHLGTF
RLLEEKTPPGIVDKFGWCTWDAFYLTVHPQGVIEGVRHLVDGGCPPGLVL
IDDGWQSIGHDSDPITKEGMNQTVAGEQMPCRLLKFQENYKFRDYVNPKA
TGPRAGQKGMKAFIDELKGEFKTVEHVYVWHALCGYWGGLRPQVPGLPEA
RVIQPVLSPGLQMTMEDLAVDKIVLHKVGLVPPEKAEEMYEGLHAHLEKV
GIDGVKIDVIHLLEMLCEDYGGRVDLAKAYYKAMTKSINKHFKGNGVIAS
MEHCNDFMFLGTEAISLGRVGDDFWCTDPSGDPNGTFWLQGCHMVHCAND
SLWMGNFIHPDWDMFQSTHPCAAFHAASRAISGGPIYVSDSVGKHNFDLL
KKLVLPDGSILRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVIGA
FNCQGGGWCRETRRNQCFSQYSKRVTSKTNPKDIEWHSGENPISIEGVKT
FALYLYQAKKLILSKPSQDLDIALDPFEFELITVSPVTKLIQTSLHFAPI
GLVNMLNTSGAIQSVDYDDDLSSVEIGVKGCGEMRVFASKKPRACRIDGE
DVGFKYDQDQMVVVQVPWPIDSSSGGISVIEYLF

HvSIP:

MTVTPQITVGDGRLAVRGRTVLSGVPDNVTAAHAAGAGLVDGAFVGATAA
EAKSHHVFTFGTLRDCRFMCLFRFKLWWMTQRMGTSGRDVPLETQFILIE

VPAAAGNDDGDSSDGDSEPVYLVMLPLLEGQFRTVLQGNDQDELQICIES
GDKAVETEQGMNNVYVHAGTNPFDTITQAVKAVEKIITQTFHHREKKTVPS
FVDWFGWCTWDAFYTDVTADGVKQGLRSLAEGGAPPRFLIIDDGWQQIGS
ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDETKK
EHGVKSVYVWHAMAGYWGGVKPSAAGMEHYEPALAYPVQSPGVTGNQPDI
VMDSLSVLGLGLVHPRRVHRFYDELHAYLAACGVDGVKVDVQNIVETLGA
GHGGRVALTRAYHRALEASVARNFPDNGCISCMCHNTDMLYSAKQTAVVR
ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFHSLHPAAEYHGA
ARAIGGCPIYVSDKPGNHNFDLLRKLVLPDGSVLRAQLPGRPTRDCLFSD
PARDGASLLKIWNMNKCAGVVGVFNCQGAGWCRVAKKTRIHDEAPGTLTG
SVRAEDVEAIAQAAGTGDWGGEAVVYAHRAGELVRLPRGATLPVTLKRLE
YELFHVCPVRAVAPGVSFAPIGLLHMFNAGGAVEECTVETGEDGNAVVGL
RVRGCGRFGAYCSRRPAKCSVDSADVEFTYDSDTGLVTADVPVPEKEMYR
CALEIRV

AmSTS:

MAPPYDPIPIPIPMSAILNFLSSTVKDNSFELLDGTLSVKNVPILTDIPS
NVSFSSFSSIVQSSEAPVPLFQRAQSLSSSGGFLGFSQNEPSSRLMNSLG
KFTDRDFVSIFRFKTWWSTQWVGTTGSDIQMETQWIMLDVPEIKSYAVVV
PIVEGKFRSALFPGKDGHILIGAESGSTKVKTSNFDAIAYVHVSENPYTL
MRDAYTAVRVHLNTFKLIEEKSAPPLVNKFGWWTWDAFYLTVEPAGIYHG
VQEFADGGLTPRFLIIDDGWQSINNDDNDPNEDAKNLVLGGTQMTARLHR
LDECEKFRKYKGGSMSGPNRPPFDPKKPKLLISKAIEIEVAEKARDKAAQ
SGVTDLARYEAEIEKLTKELDQMFGGGGEETSSGKSCSSCSCKSDNFGMK
AFTKDLRTNFKGLDDIYVWHALAGAWGGVRPGATHLNAKIVPTNLSPGLD
GTMTDLAVVKIIEGSTGLVDPDQAEDFYDSMHSYLSSVGITGVKVDVIHT
LEYISEDYGGRVELAKAYYKGLSKSLAKNFNGTGLISSMQQCNDFFLLGT
EQISMGRVGDDFWFQDPNGDPMGVYWLQGVHMIHCAYNSMWMGQFIQPDW
DMFQSDHPGGYFHAGSRAICGGPVYVSDSLGGHNFDLLKKLVFNDGTIPK
CIHFALPTRDCLFKNPLFDSKTILKIWNFNKYGGVIGAFNCQGAGWDPKE
QRIKGYSQCYKPLSGSVHVSGIEFDQKKEASEMGEAEEYAVYLSEAEKLS
LATRDSDPIKITIQSSTFEIFSFVPIKKLGEGVKFAPIGLTNLFNAGGTI
QGLVYNEGIAKIEVKGDGKFLAYSSVVPKKAYVNGAEKVFAWSGNGKLEL
DITWYEECGGISNVTFVY

PsSTS-1:

MAPPLNSTTSNLIKTESIFDLSERKFKVKGFPLFHDVPENVSFRSFSSIC
KPSESNAPPSLLQKVLAYSHKGGFFGFSHETPSDRLMNSIGSFNGKDFLS
IFRFKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIIPIIEKCFRS
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYSAIR
VHLNSFRLLEEKTIPNLVDKFGWCTWDAFYLTVNPIGIFHGLDDFSKGGV

EPRFVIIDDGWQSISFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPNNFTDLILKGIEHEKLRKKREEAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSGEKSEMKSEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQHCNDFFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVCAKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTVLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTEVEWDQKEETSHLGKAEEYVVYLNQAEELSLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSESPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVSDME
IFF

PsSTS-2:
MAPPLNSTTSNLIKTESIFDLSERKFKVKGFPLFHDVPENVSFRSFSSIC
KPSESNAPPSLLQKVLAYSHKGGFFGFSHETPSDRLMNSLGSFNGKDFLS
IFRFKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIIPIIEKCFRS
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYIAIR
VHLNSFRLLEEKTIPNLVDKFGWCTWDAFYLTVNPIGIFHGLDDFSKGGV
EPRFVIIDDGWQSISFDGCDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPKKFTDLILKGIEHEKLRKKREEAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSVEKSEMKSEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKFVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQQCNDFFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFKSDHVCAKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTLLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTQVEWDQKEETSHFGKAEEYVVYLNQAEELCLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSESPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVS

SaSTS:
MAPPNDPISSIFSPLISVKKDNAFELVGGKLSVKNVPLLSEIPSNVTFKS
FSSICQSSGAPAPLYNRAQSLSNCGGFLGFSQKESADSVTNSLGKFTNRE
FVSIFRFKTWWSTQWVGTSGSDIQMETQWIMLNLPEIKSYAVVIPIVEGK
FRSALFPGKDGHVLISAESGSTCVKTTSFTSIAYVHVSDNPYTLMKDGYT
AVRVHLDTFKLIEEKSAPPLVNKFGWCTWDAFYLTVEPAGIWNGVKEFSD
GGFSPRFLIIDDGWQSINIDGQDPNEDAKNLVLGGTQMTARLHRFDECEK
FRKYKGGSMMGPKVPYFDPKKPKLLISKAIEIEGVEKARDKAIQSGITDL

SQYEIKLKKLNKELDEMFGGGGNDEKGSSKGCSDCSCKSQNSGMKAFTND
LRTNFKGLDDIYVWHALAGAWGGVKPGATHLNAKIEPCKLSPGLDGTMTD
LAVVKILEGSIGLVHPDQAEDFYDSMHSYLSKVGITGVKVDVIHTLEYVS
ENYGGRVELGKAYYKGLSKSLKKNFNGSGLISSMQQCNDFFLLGTEQISM
GRVGDDFWFQDPNGDPMGVFWLQGVHMIHCAYNSMWMGQIIHPDWDMFQS
DHCSAKFHAGSRAICGGPVYVSDSLGGHDFDLLKKLVFNDGTIPKCIHFA
LPTRDCLFKNPLFDSKTILKIWNFNKYGGVVGAFNCQGAGWDPKEQRIKG
YSECYKPLSGSVHVSDIEWDQKVEATKMGEAEEYAVYLTESEKLLLTTPE
SDPIPFTLKSTTFEIFSFVPIKKLGQGVKFAPIGLTNLFNSGGTIQGVVY
DEGVAKIEVKGDGKFLAYSSSVPKRSYLNGEEVEYKWSGNGKVEVDVPWY
EECGGISNITFVF

VaSTS:
MAPPNDPVNATLGLEPSEKVFDLSDGKLTVKGVVLLSHVPENVTFSSFSS
ICVPRDAPSSILQRVTAASHKGGFLGFSHVSPSDRLINSLGSFRGRNFLS
IFRFKTWWSTQWVGNSGSDLQMETQWILIEVPETESYVVIIPIIEKSFRS
ALHPGSDDHVKICAESGSTQVRASSFGAIAYVHVAETPYNLMREAYSALR
VHLDSFRLLEEKTVPRIVDKFGWCTWDAFYLTVNPVGVWHGLKDFSEGGV
APRFVVIDDGWQSVNFDDEDPNEDAKNLVLGGEQMTARLHRFEEGDKFRK
YQKGLLLGPNAPSFNPETIKELISKGIEAEHLGKQAAAISAGGSDLAEIE
LMIVKVREEIDDLFGGKGKESNESGGCCCKAAECGGMKDFTTDLRTEFKG
LDDVYVWHALCGGWGGVRPGTTHLDSKIIPCKLSPGLVGTMKDLAVDKIV
EGSIGLVHPHQANDLYDSMHSYLAQTGVTGVKIDVIHSLEYVCEEYGGRV
EIAKAYYDGLTNSIIKNFNGSGIIASMQQCNDFFFLGTKQIPFGRVGDDF
WFQDPNGDPMGVFWLQGVHMIHCSYNSLWMGQIIQPDWDMFQSDHECAKF
HAGSRAICGGPVYVSDSVGSHDFDLIKKLVFPDGTVPKCIYFPLPTRDCL
FRNPLFDQKTVLKIWNFNKYGGVIGAFNCQGAGWDPKGKKFKGFPECYKA
ISCTVHVTEVEWDQKKEAEHMGKAEEYVVYLNQAEVLHLMTPVSEPLQLT
IQPSTFELYNFVPVEKLGSSNIKFAPIGLTNMFNSGGTIQELEYIEKDVK
VKVKGGGRFLAYSTQSPKKFQLNGSDAAFQWLPDGKLTLNLAWIEENDGV
SDLAIFF

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; http://www.sdc.co.jp/genetyx/) with default parameters. In the molecular phylogenic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

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Sc-04 (full-length):
MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS
LQPGLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM
HLGTFKLLEEKTAPVIIDKFGWCTWDAFYLKVHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAICHDEDPITDQEGMKRTSAGEQMPCRLVKLEENYKFRQ
YCSGKDSEKGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLYEGLHSRLES
AGIDGVKVDVIHLLEMLSEEYGGRVELAKAYYKALTASVKKHFKGNGVIA
SMEHCNDFFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY
NSLWMGNFIQPDWDMFQSTHPCAEFHAASRAISGGPVYVSDCVGKHNFKL
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKTMLKIWNLNKYTGVLG
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LFNCQGGGWCPVTRRNKSASEFSQTVTCLASPQDIEWSNGKSPICIKGMN
VFAVYLFKDHKLKLMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP
IGLVNMLNTGGAIQSMEFDNHIDVVKIGVRGCGEMKVFASEKPVSCKLDG
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6th day of September, 2005

Akitsu NAGASAWA

Table 1

Code	Protein*	Organism	Accession**	Reference	Author/Assignee
Sc-03	RFS	***Beta vulgaris***	E37133	09/301,766	Sumitomo Chemical
Sc-05	RFS	***Brassica juncea***	E36417	09/301,766	Sumitomo Chemical
Sc-02	RFS	***Vicia faba***	E24423	08/992,914	Sumitomo Chemical
Sc-04	RFS	***Glycine max***	E24424	08/992,914	Sumitomo Chemical
Aj-05	RFS	***Cucumis sativus***	AF073744	Family GH36***	Ohsumi et al.
PsRFS	RFS	***Pisum sativum***	AJ426475	Family GH36	Peterbauer et al.
HvSIP	SIP	***Hordeum vulgare***	M77475	Family GH36	Heck et al.
PsSTS-1	STS	***Pisum sativum***	AJ311087	Family GH36	Peterbauer et al.
PsSTS-2	STS	***Pisum sativum***	AJ512932	Family GH36	Peterbauer et al.
VaSTS	STS	***Vigna angularis***	Y19024	Family GH36	Peterbauer et al.
AmSTS	STS	***Alonsoa meridionalis***	AJ487030	Family GH36	Voitsekhovskaja
SsSTS	STS	***Stachys affinis***	AJ344091	Family GH36	Pesch and Schmitz

*Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

**Accession: GenBank Accession Number.

***Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: http://afmb.cnrs-mrs.fr/CAZY/GH_36.html)

Table 2

Code	Sc-02	Sc-03	Sc-04	Sc-05	PsRFS	Aj-05	HvSIP	AmSTS	PsSTS-1	PsSTS-2	SaSTS	VaSTS
amino acids	799	783	611	777	798	784	757	868	853	847	863	857
Sc-02		62	54	62	89	64	39	43	43	42	42	43
Sc-03			50	63	63	66	39	42	43	42	42	43
Sc-04				50	54	50	29	35	35	35	34	36
Sc-05					62	70	39	44	44	43	44	45
PsRFS						64	38	42	43	43	42	43
Aj-05							38	42	44	43	43	43
HvSIP								33	34	34	34	34
AmSTS									64	64	83	64
PsSTS-1										98	65	75
PsSTS-2											65	74
SaSTS												65
VaSTS												

Table 3

Code	Sc-02	Sc-03	Sc-04	Sc-05	PsRFS	Aj-05	HvSIP	AmSTS	PsSTS-1	PsSTS-2	SaSTS	VaSTS
amino acids	799	783	781	777	798	784	757	868	853	847	863	857
Sc-02		61	75	61	88	63	41	52	50	50	49	50
Sc-03			65	63	62	66	40	49	39	39	49	49
Sc-04				67	75	65	38	39	50	43	51	52
Sc-05					60	70	40	51	51	51	52	50
PsRFS						65	40	51	49	49	51	50
Aj-05							39	49	50	50	50	50
HvSIP								38	38	38	38	38
AmSTS									63	64	81	62
PsSTS-1										96	63	72
PsSTS-2											64	72
SaSTS												64
VaSTS												

Table 4

Code	Sc-02	Sc-03	Sc-04	Sc-05	PsRFS	Aj-05	HvSIP	AmSTS	PsSTS-1	PsSTS-2	SaSTS	VaSTS
amino acids	799	783	781	777	798	784	757	868	853	847	863	857
Sc-02		61	75	61	88	63	41	43	42	42	41	42
Sc-03			64	63	62	66	40	41	42	42	41	42
Sc-04				67	75	65	38	42	43	44	42	46
Sc-05					60	70	40	45	45	45	45	45
PsRFS						65	40	42	42	42	42	42
Aj-05							39	41	43	43	42	43
HvSIP								33	35	35	33	34
AmSTS									62	62	78	62
PsSTS-1										96	63	73
PsSTS-2											64	73
SaSTS												64
VaSTS												

Fig. 1

[GENETYX : Evolutionary tree]
Date : 2004.2.4
Method: UPGMA